154 Platelets

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Definition

The platelet is a circulating anuclear fragment of a bone marrow megakaryocyte, 3 to 4 μm in diameter, with limited synthetic capability. The mean normal platelet count is between 250,000 and 260,000 cells/mm^3, although there is a wide range of accepted normal values in most laboratories that extend as low as 150,000 to as high as 400,000/mm^3.

Technique

With recent technologic advances, accurate platelet counts are now widely available as a component of automated blood counts. The counts are done in a few seconds using either an electronic particle counting method (e.g., Coulter S-plus) or an optical method (e.g., Ortho ELT 8). This contrasts with the 30 to 45 minutes it takes to do a count using a hemocytometer. The new automated counters have only a 4% coefficient of variation in the normal range and are thus more reproducible than the microscopic method. The coefficient of variation increases to 10% for counts less than 50,000/mm^3, making it critical to examine the peripheral smear when the count is in this range and perform a manual count if a therapeutic decision is to be made based on the count.

The normal ratio of platelets to red blood cells on a peripheral smear is approximately 1:20; thus a high-power microscopic field normally contains 8 to 10 platelets. If one platelet per high-power field is not seen, the platelet count is usually below 20,000/mm^3, a level consistent with a high probability of spontaneous bleeding.

Both unusually large- and small-sized platelets can increase the error inherent in the automated instruments. Underestimates are particularly seen using electronic particle counters that have prefixed thresholds in patients with immune thrombocytopenia, myeloproliferative disorders, and certain inherited disorders such as Bernard–Soulier syndrome. Sample preparation is critical for automated counting methods. Platelet aggregation, usually due to poor collection techniques and platelet satellitism to leukocytes in ethylenediaminetetraacetic acid (EDTA) collected blood, is a frequent cause of count underestimation. It is therefore essential to screen blood smears to verify platelet count in all patients.

A second technologic advance provided with many new electronic counters is the measurement of mean platelet volume (MPV). It is obtained by measuring a histogram of platelet volumes, with normal subjects having a mean value of 8.5 to 9.0 femtoliters. Controversy exists about the accuracy and thus the usefulness of the MPV, especially when EDTA collected blood is used, because EDTA causes significant platelet swelling over time at room temperature. In normal persons, the MPV varies inversely but nonlinearly with the platelet count. Within each laboratory, then, using standardized assay methods, the MPV can give semiquantitative information in the evaluation of the thrombocytopenic patient. Patients with acute leukemia, aplastic anemia, or drug-induced marrow hypoplasia have a normal or low MPV, whereas those with a peripheral platelet destructive process and a healthy marrow (such as is seen in immune thrombocytopenia or disseminated intravascular coagulation) have an elevated MPV. Unfortunately, the accuracy of the MPV is limited to counts greater than 20,000/mm^3, making it useless in the evaluation of the patient group most likely to benefit from this information. It is unlikely that this determination will ever be more valuable than a careful inspection of platelet size on a peripheral smear.

Basic Science

Platelet Function

Platelets function by maintaining the integrity of the vascular tree, producing the platelet plug in the first phase of clotting, and by producing platelet factor 3, an essential component of the coagulation cascade. Clotting is initiated by interruption of normal vascular integrity. This exposes circulating platelets to subendothelial collagen and multimers of factor VIII (von Willebrand factor), which leads to a shape change in the platelets from discoid to spheroid, and their adhesion locally, the first step in the coagulation process. Adenosine diphosphate (ADP) and thrombin are then released from the activated platelets and thromboxane A_2 is generated, causing platelet aggregation. The platelet release reaction follows with the secretion of fibrinogen, von Willebrand factor, factor V, platelet factor 4, and B-thromboglobulin from the alpha granules and serotonin, calcium ions, ADP, and adenosine triphosphate (ATP) from the dense bodies. This forms the platelet plug. Platelet coagulant activity is then produced, primarily through platelet factor 3, which ultimately, with the plasma clotting factors, leads to the formation of the fibrin clot, the final step in the coagulation sequence.

Megakaryocytopoiesis

Megakaryocytes constitute less than 0.1% of the marrow cell population and go through defined stages of development before platelets are produced. Megakaryoblasts have compact but lobed nuclei and a thin rim of basophilic cytoplasm, and range up to 20 μm in size. Polyploidization or endomitosis with up to the 32N ploidy stage takes place only in this cell. In the promegakaryocyte, azurophilic granules begin to be seen and the nucleus becomes horseshoe shaped. The

next stage in development is the granular megakaryocyte, characterized by multilobed nucleus, pink cytoplasm, size up to 50 μm, and large numbers of granules. The final stage of development, the mature megakaryocyte, is characterized by the liberation of platelets into the sinusoidal spaces. Each megakaryocyte produces between 1000 and 1500 platelets, which circulate for an average of only 9 days because of their limited synthetic capacity.

Platelet production appears to be mediated by soluble factors at two levels. The first acts at the stem cell level and the second on maturing megakaryocytes. It is possible to grow megakaryocytes in vitro from either blood or bone marrow progenitors, which are called CFU-Mega (colony forming unit-megakaryocyte). Such assays appear to require MegaCSF, a specific colony stimulating factor, obtained from sera or plasma of patients with aplastic anemia, or supernatant media from phytohemagglutinin-stimulated lymphocytes. With increased production of this mediator the population of progenitor cells is stimulated to divide, resulting in the production of more megakaryocytes. It has, however, no further role in platelet production. In acute thrombocytopenia the size of the megakaryocytes and the ploidy number increase, mediated by the second stimulatory agent, named thrombopoietin. This molecule has a limited spectrum of activity, acting only on maturing megakaryocytes, that is, it does not stimulate the production of colony formation in vitro. In addition, thrombocytosis, which decreases thrombopoietin concentration, does not cause a complete inhibition of marrow megakaryocyte progenitors.

Thrombocytopenic States

Thrombocytopenia is due to either decreased marrow production, increased peripheral utilization, or increased splenic sequestration. Often there are multiple causes, such as in disseminated intravascular coagulation (DIC) where there may be a nonimmune destruction as well as an inhibition of marrow production. The various etiologies are shown in Table 154.1. A bone marrow biopsy is necessary to document decreased production when the count is below 50,000/mm^3. Marrow aspirate smears are helpful if adequate megakaryocytes are seen, but decreased numbers may be seen on diluted specimens and need to be verified by a biopsy. The most common causes of decreased marrow production of platelets are drugs, infections, and either primary or secondary marrow malignancies. Because of the generally nonspecific toxic effect of the inciting agent, in most cases thrombocytopenia is accompanied by anemia and leukopenia. If secondary to a drug, withdrawal of the drug leads to a slow count recovery that can take a month or longer. This is in contrast to the rapid recovery (several days to 2 weeks) when the drug-induced mechanism is increased peripheral destruction.

Thrombocytopenia associated with a shortened platelet survival is usually due to an antibody-mediated process that may be secondary to drugs, quinidine being the most common; neoplasms, usually lymphoid; connective tissue disorders such as lupus; or idiopathic thrombocytopenic purpura (ITP). Drugs such as quinidine act as haptens that bind to a plasma protein and elicit the production of an IgG antiplatelet antibody. The antibody-coated platelet is then removed by the reticuloendothelial (RE) system. Other drugs form drug-antibody complexes that are adsorbed nonspecifically to the platelet surface and are removed by the RE system.

Table 154.1
Differential Diagnosis of Thrombocytopenia

- Decreased production
  - Primary bone marrow disorders
    - Congenital disorders
      - Fanconi's anemia
      - Dyskeratosis congenita
      - Paroxysmal nocturnal hemoglobinuria
      - Wiskott-Aldrich syndrome
      - May-Hegglin anomaly
      - Chediak-Higashi anomaly
      - Cyclic thrombocytopenia
    - Primary bone marrow malignancies
      - Acute and chronic myeloid and lymphoid leukemia
      - Multiple myeloma
      - Hairy cell leukemia
    - Myelofibrosis
    - Myelodysplastic syndrome
    - Aplastic anemia
  - Drugs and chemicals
    - Alcohol
    - Thiazide diuretics
    - Chemotherapeutic agents
    - Benzene
    - Estrogens
    - Gold salts
    - Chloramphenicol
  - Infections
    - Bacterial sepsis
    - Viral infections
  - Radiation
  - Marrow infiltration
  - Nutritional
    - B_{12} or folate deficiency
    - Iron deficiency
- Increased peripheral utilization
  - Nonimmune
    - Hemangiomas
    - Atrial myxomas
    - Disseminated intravascular coagulation
    - Hemolytic uremic syndrome/thrombotic thrombocytopenic purpura
    - Prosthetic valves, grafts, and bypass pumps
  - Immune
    - Drugs
      - Quinine and quinidine
      - Heparin
      - Antimicrobials
    - Connective tissue disorders
    - Lymphoid neoplasms
    - Posttransfusion purpura
    - Isoimmune neonatal thrombocytopenia
    - Idiopathic thrombocytopenic purpura
- Increased sequestration—hypersplenism
- Dilutional—massive blood transfusion

Acute ITP is characterized by rapid onset of severe thrombocytopenia, usually in a child with an antecedent viral infection in up to 80% of the cases. It resolves, usually without therapy, in 4 to 6 weeks. The etiology is presumably due to immune complexes of viral antigen and antibody that are absorbed onto the platelet surface, which are then removed by the RE system. Chronic ITP is characteristically seen in females between 30 and 50 years and is characterized by elevated platelet-bound IgG in over 90% of patients. Serum antiplatelet antibodies are seen in only 50% of patients. The spleen not only produces the autoantibody but is the main site of platelet sequestration. Since this is an IgG-mediated process and IgG crosses the placenta, pregnant women with ITP are at risk of delivering thrombocytopenic babies.

In the steady state one-third of the platelet mass exists as a freely exchangeable pool in the spleen. Thus in hypersplenism, usually on the basis of chronic liver disease, thrombocytopenia is due to increased pooling in the spleen and is usually accompanied by increased marrow megakaryocytes. The thrombocytopenia is characteristically mild in the presence of a healthy marrow.

Thrombocytosis

As shown in Table 154.2, the causes of thrombocytosis are primary or secondary marrow overproduction, or the transient effect of decreased sequestration seen after splenectomy. While thrombocytosis is defined as a count above 450,000/mm^3, symptoms are rare if the count is less than 1,000,000/mm^3. The most common cause of a count above 1,000,000/mm^3 is a myeloproliferative disorder, although counts this high are sometimes seen following splenectomy, especially if done for ITP. The thrombocytosis seen after splenectomy for nonhematologic diseases usually resolves in 2 months and does not cause symptoms. While not seen with secondary causes of thrombocytosis, the elevated counts associated with myeloproliferative disorders are often accompanied by increased bleeding tendencies. Functional abnormalities are seen in these platelets as measured by abnormal bleeding times, abnormal platelet aggregation in vitro, and abnormal platelet factor 3 release.

Qualitative Platelet Abnormalities

A variety of acquired disorders are associated with qualitative platelet disorders, usually causing a bleeding diathesis. Uremia causes a complicated picture of abnormal platelet aggregation, abnormal levels of platelet factor 3, and abnormal platelet retention on glass beads, none of which is consistent. Bleeding is uncommon, however, as these abnormalities are reversible with dialysis. Liver disease, dysproteinemias, acute leukemia, and many drugs cause similar abnormalities. Aspirin is the most common cause of a qualitative platelet abnormality. It acts by inhibiting cyclooxygenase, preventing the production of thromboxane A_2, which is necessary for platelet aggregation and secretion. Other prostaglandin inhibitors, such as ibuprofen, indomethacin, and phenylbutazone, also inhibit platelet aggregation but, unlike aspirin, which binds to platelets irreversibly, the effects of these agents occur only in the presence of active drugs.

A variety of inherited disorders are associated with qualitative platelet function defects and bleeding tendencies. In the Bernard–Soulier syndrome, there is decreased platelet adherence to the subendothelial multimers of VIII: von Willebrand factor. In Glanzmann's thrombasthenia, there are absent or abnormal surface glycoproteins, which lead to impaired platelet aggregation to ADP and fibrinogen.

Table 154.2
Differential Diagnosis of Thrombocytosis

Primary bone marrow disorders
Myeloproliferative disorders
Polycythemia vera
Myelofibrosis
Essential thrombocythemia
Chronic myelogenous leukemia
Bone marrow stimulation
Acute and chronic infections
Autoimmune disorders
Neoplasms
Rebound states
Following chemotherapy
Alcohol withdrawal
Treatment of B_{12} or folate deficiency
After hemorrhage or surgery
Iron deficiency anemia
Decreased sequestration following splenectomy

The Platelet in Atherosclerosis

Platelets appear to be important in the pathophysiology of atherosclerosis. They do not seem to initiate the disorder because they do not spontaneously attach to intact epithelium. They may, however, adhere to endothelial surfaces damaged possibly by hypertension, smoking, and/or abnormal dietary lipids through their binding to exposed collagen in the subendothelium. After binding, they cause further platelet aggregation, initiate the platelet release reaction, and release platelet-driven growth factor. Ultimately, smooth muscle proliferation and lipid accumulation result, the characteristic features of atherosclerosis. Disordered blood flow locally around these focal lesions then leads to further platelet aggregation and to release of ADP, thromboxane A_2, and serotonin, which can ultimately lead to complete thrombosis and/or spasm of the narrowed blood vessels.

Clinical Significance

The approach to thrombocytopenia depends on its etiology. Examination of the peripheral smear will provide verification of the low count as well as the platelet size. In addition to looking for signs of bleeding on the physical examination, the spleen should be palpated to evaluate for possible sequestration. A bone marrow examination is generally recommended, particularly if the thrombocytopenia is severe and a treatment decision is to be made. In disorders of decreased marrow production, the platelet size is normal or smaller and the marrow shows decreased megakaryocytes. In disorders of increased peripheral destruction, there is a normal to increased platelet size and increased megakaryocytes. If evidence for peripheral destruction is found, clotting tests should be done to evaluate for a consumptive coagulopathy.

Bleeding due to thrombocytopenia is related to two factors: the absolute count and the rapidity of the decline. The risk of significant bleeding increases dramatically below a count of 20,000/mm^3, and the risk of spontaneous CNS hemorrhage increases dramatically at counts below 10,000/mm^3. Bleeding usually takes the form of petechiae of the skin, usually of the lower extremities, bleeding from the oral or nasal mucus membranes, or, less commonly, the bladder. Less frequent but potentially more significant is the risk of retinal hemorrhages, which may be the first sign of bleeding and seem to occur more frequently in severely anemic patients. These hemorrhages are usually seen near the disc, and often present as a blind spot, but can also affect the macula, severely impeding vision. Infection and fever in general increase the risk of bleeding in the thrombocytopenic patient at counts up to 50,000/mm^3. If significant bleeding occurs at a count above 50,000/mm^3, other

etiologies, such as a coagulation abnormality or vascular defect, should be investigated, as spontaneous bleeding from thrombocytopenia alone at these counts is uncommon. Platelet counts in the 5000 to 20,000/mm³ range in patients with ITP are often not associated with bleeding because the platelets are younger and larger than normal and have an enhanced hemostatic effect.

Platelet transfusions, usually from random donors, are the treatment of choice for the prophylaxis and treatment of bleeding due to thrombocytopenia of decreased marrow production. The number of platelets obtained from a unit of blood when transferred into a nonalloimmunized patient should raise the count approximately 10,000/mm³. The half-life of transfused platelets, under ideal conditions, is the same as that for normal endogenous platelets, 9 days. It is customary to transfuse patients empirically who have decreased marrow production of platelets, usually drug-induced, with 4 to 6 units of random donor platelets whenever the count drops below 20,000/mm³. Some available data suggest that such prophylactic therapy is not associated with enhanced survival in leukemic patients when compared to those who are transfused for bleeding only. While few would disagree with prophylactic platelet transfusions for counts below 10,000/mm³ because of the increased risk of fatal CNS bleeding, it may be reasonable to observe inpatients closely without prophylactic transfusions at counts above 10,000, and transfuse only for bleeding or conditions associated with an increased risk of bleeding such as an abrupt fall in counts or sepsis. It is prudent to minimize transfusions of platelets because the risk of non-A, non-B hepatitis is as high as 40% for those receiving many transfusions, with as many as 50% of these developing chronic liver disease.

A minimum of one-half of patients with acute leukemia who receive repeated platelet transfusions ultimately become alloimmunized to random donor platelets. Lymphocytotoxic antibodies against HLA antigens may mark for this refractoriness, and their determination is valuable for previously transfused patients in whom future transfusions are expected. If the test is positive, single donor platelets from family members or HLA-matched platelets will be necessary to procure for thrombocytopenic episodes. Another alternative is to plateletpherese the patient during periods when the count is normal and cryopreserve the platelets. If matched platelets are not available for an alloimmunized bleeding patient, the transfusion of 20 or more random donor units is usually effective in temporarily stopping the bleeding. Patients with ITP or DIC, even with a healthy marrow, should also be given platelets for severe bleeding episodes. Epsilon-aminocaproic acid at doses of approximately 12 g per day in divided doses has occasionally been of value in the prophylaxis of thrombocytopenic patients who are alloimmunized. Such therapy is, however, quite expensive.

While no therapy is recommended for children with acute ITP because of its short duration, corticosteroids with an initial prednisone dose of 1 mg/kg per day are the initial therapy for patients with chronic ITP. A beneficial effect usually begins within the first 3 to 4 days. A therapeutic effect (i.e., a count above 50,000) is seen in 60 to 70% of patients usually by 3 to 4 weeks after therapy is started. A higher prednisone dose of 1.5 to 2.0 mg/kg/day may be effective in a slightly higher proportion of patients. Steroids appear to work in a variety of ways. They reduce phagocytosis of the antibody-coated platelets by the RE system, may actually inhibit the binding of antibody to the platelet, increase the catabolism of IgG, and may depress autoantibody production. Once a beneficial effect is seen, steroids are continued at full doses for a month and then are gradually tapered over 4 to 6 months. If a count in the 50,000/mm³ range cannot be maintained by less than 20 mg of prednisone per day, a splenectomy is recommended. A splenectomy causes a rise in the platelet count, usually in the first few days, in approximately 70% of patients. Unlike autoimmune hemolytic anemia, radioisotopic studies are of no value in determining which patients will benefit from splenectomy. For patients refractory to both steroids and splenectomy, immunosuppressive therapy with azothioprine is of value in about one-third, but 2 months of therapy are needed to determine a beneficial effect. High-dose intravenous gamma globulin at a dosage of 400 mg/kg/day for 5 days increases the platelet count as soon as 24 hours in up to 80% of patients with ITP. It is felt temporarily to block the RE system from phagocytosing antibody-coated platelets. While the effect usually lasts only a month or less, it may permit a safe splenectomy or other surgical procedures for patients in whom steroids are either contraindicated or ineffective. Because of its high cost and transient effect, it should not be used as routine therapy for the newly diagnosed patient.

Patients with secondary thrombocytosis usually do not develop symptoms. Those with a myeloproliferative disorder may develop signs and symptoms of thrombosis or ischemia, which may affect the peripheral vasculature or the CNS. Therapy is directed at reducing platelet production and inhibiting platelet aggregation in the microvasculature. The precise therapy varies depending on the disorder, but hydroxyurea or oral alkylating agents are usually effective in decreasing platelet production. Aspirin at a dosage of 300 mg/day is often given as well. In life-threatening cases, plateletpheresis can reduce the count rapidly. Extreme care must be taken to assure a normal blood pressure during the procedure as hypotension may potentiate the risk of acute vascular occlusion.

References

Dumoulin-Lagrange M, Capelle C. Evaluation of automated platelet counters for the enumeration and sizing of platelets in the diagnosis and management of hemostatic problems. Semin Throm Hemos 1983;9:235–44.

Eisenstaedt R. Blood component therapy in the treatment of platelet disorders. Semin Hematol 1986;23:2–7.

Gewirtz AM. Human megakaryocutopoiesis. Semin Hematol 1986;23:27–42.

Holmsen H. Platelet metabolism and activation. Semin Hematol 1985;22:219–40.

Karpatkin S. Autoimmune thrombocytopenic purpura. Semin Hematol 1985;22:260–88.

Murphey S, Litwin S, Herring LM, et al. Indications for platelet transfusion in children with acute leukemia. Am J Hematol 1982;12:347–56.

Packham MA, Mustard JF. The role of platelets in the development and complications of atherosclerosis. Semin Hematol 1986;23:8–26.

*Thompson AR, Harker LA. Quantitative platelet disorders. In: Manual of hemostasis and thrombosis, 3d ed. Philadelphia: F. A. Davis, 1983;65–85.